Fig. 1

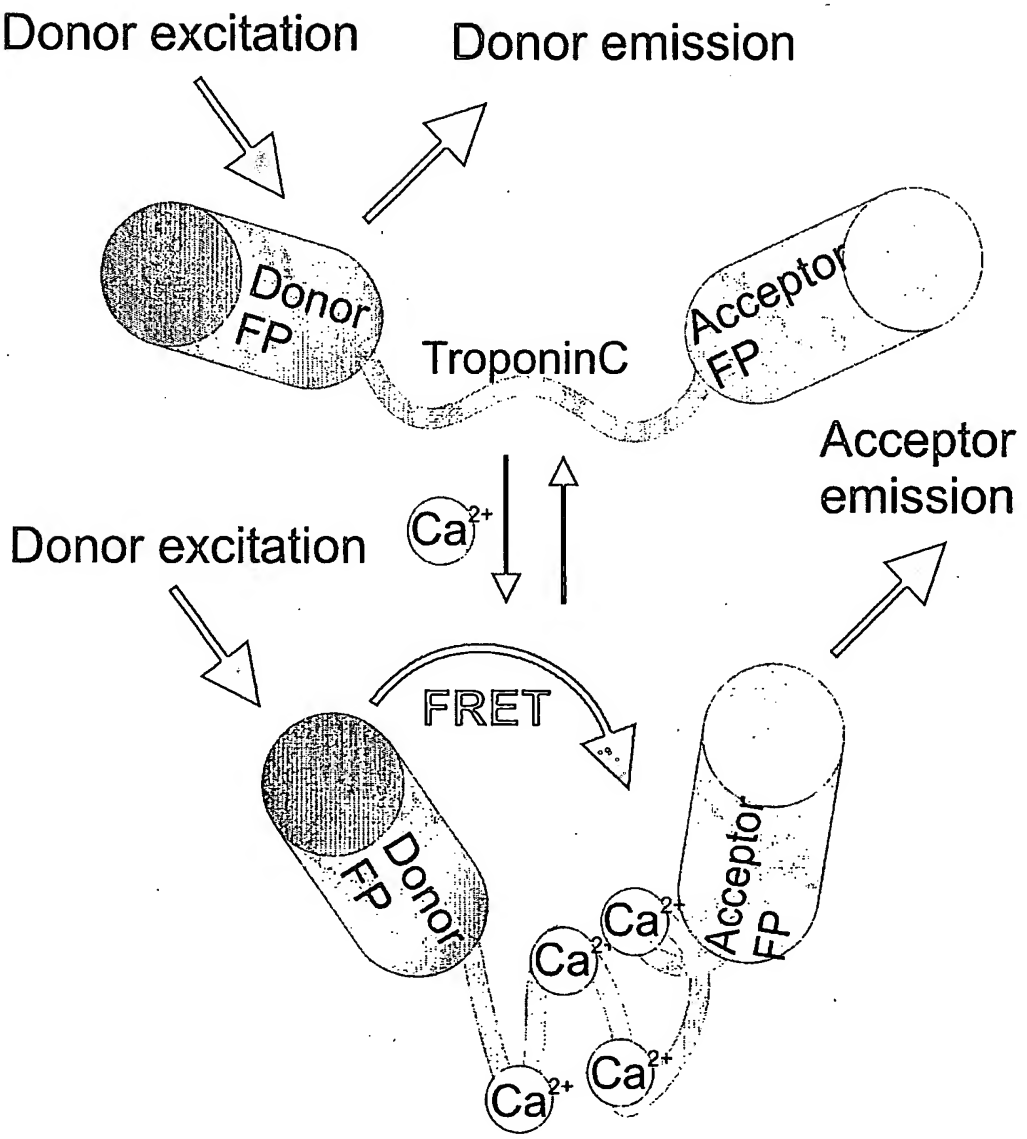

Donor excitation
Donor emission
Donor FP
TroponinC
Acceptor FP
Acceptor emission
Donor excitation
Ca2+
FRET
Donor FP
Acceptor FP
Ca2+
Ca2+
Ca2+
Ca2+

Fig. 2

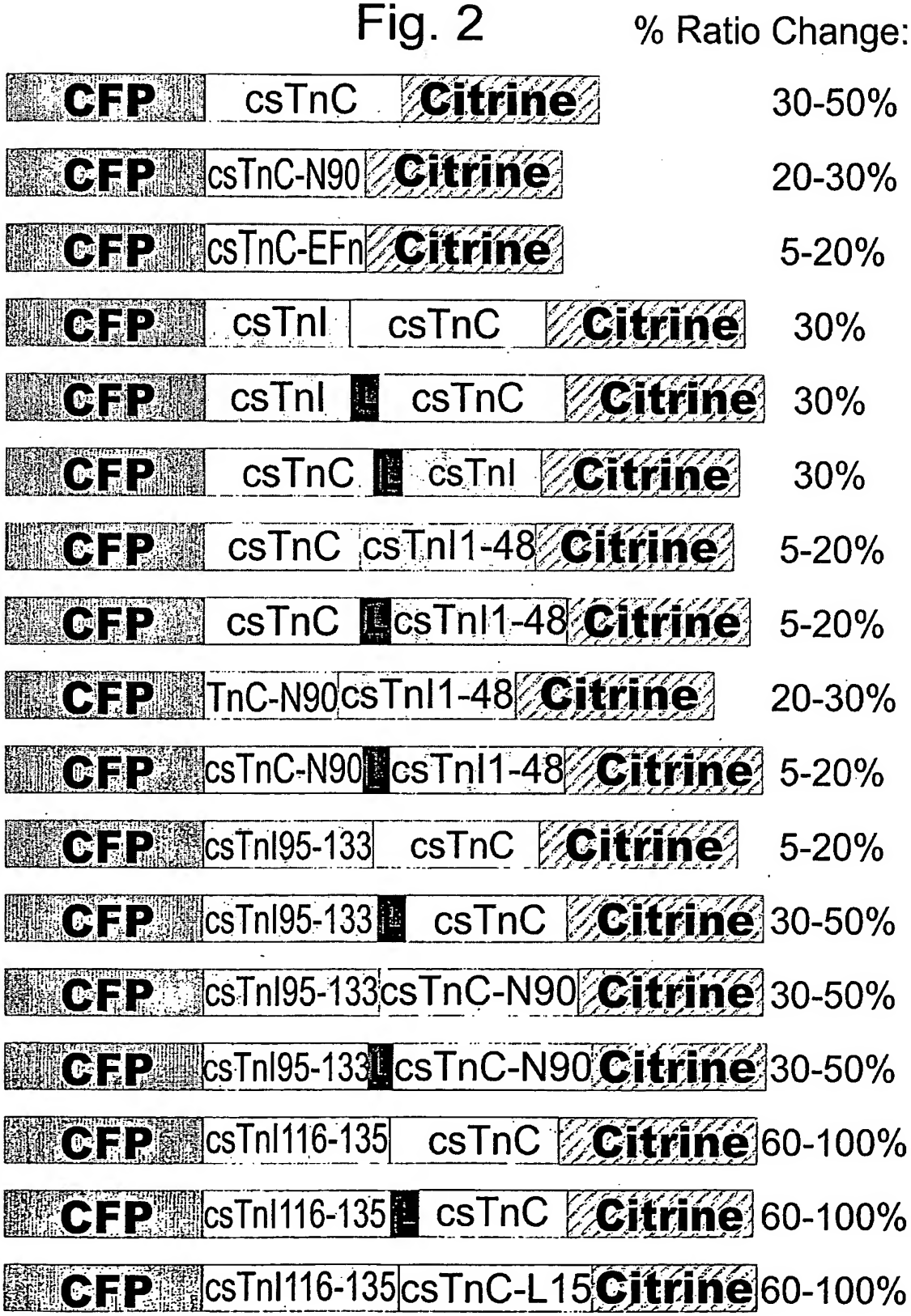

% Ratio Change:
CFP csTnC Citrine 30-50%
CFP csTnC-N90 Citrine 20-30%
CFP csTnC-EFn Citrine 5-20%
CFP csTnI csTnC Citrine 30%
CFP csTnI L csTnC Citrine 30%
CFP csTnC L csTnI Citrine 30%
CFP csTnC csTnI1-48 Citrine 5-20%
CFP csTnC L csTnI1-48 Citrine 5-20%
CFP TnC-N90 csTnI1-48 Citrine 20-30%
CFP csTnC-N90 L csTnI1-48 Citrine 5-20%
CFP csTnI95-133 csTnC Citrine 5-20%
CFP csTnI95-133 L csTnC Citrine 30-50%
CFP csTnI95-133 csTnC-N90 Citrine 30-50%
CFP csTnI95-133 L csTnC-N90 Citrine 30-50%
CFP csTnI116-135 csTnC Citrine 60-100%
CFP csTnI116-135 L csTnC Citrine 60-100%
CFP csTnI116-135 csTnC-L15 Citrine 60-100%

Fig. 2 (continued)

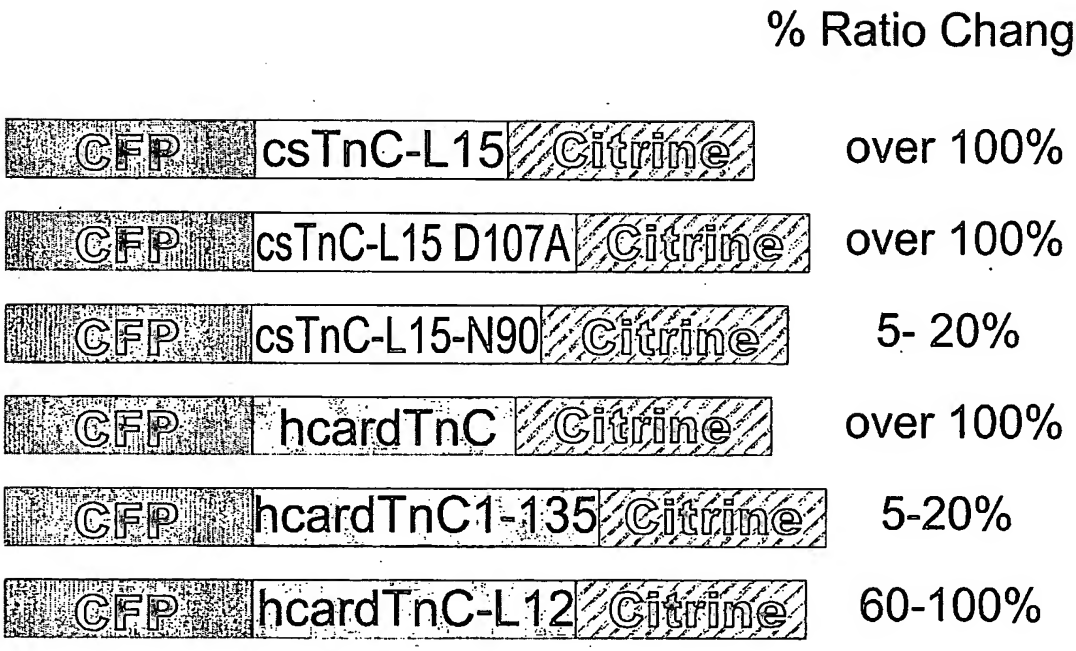


Fig. 3

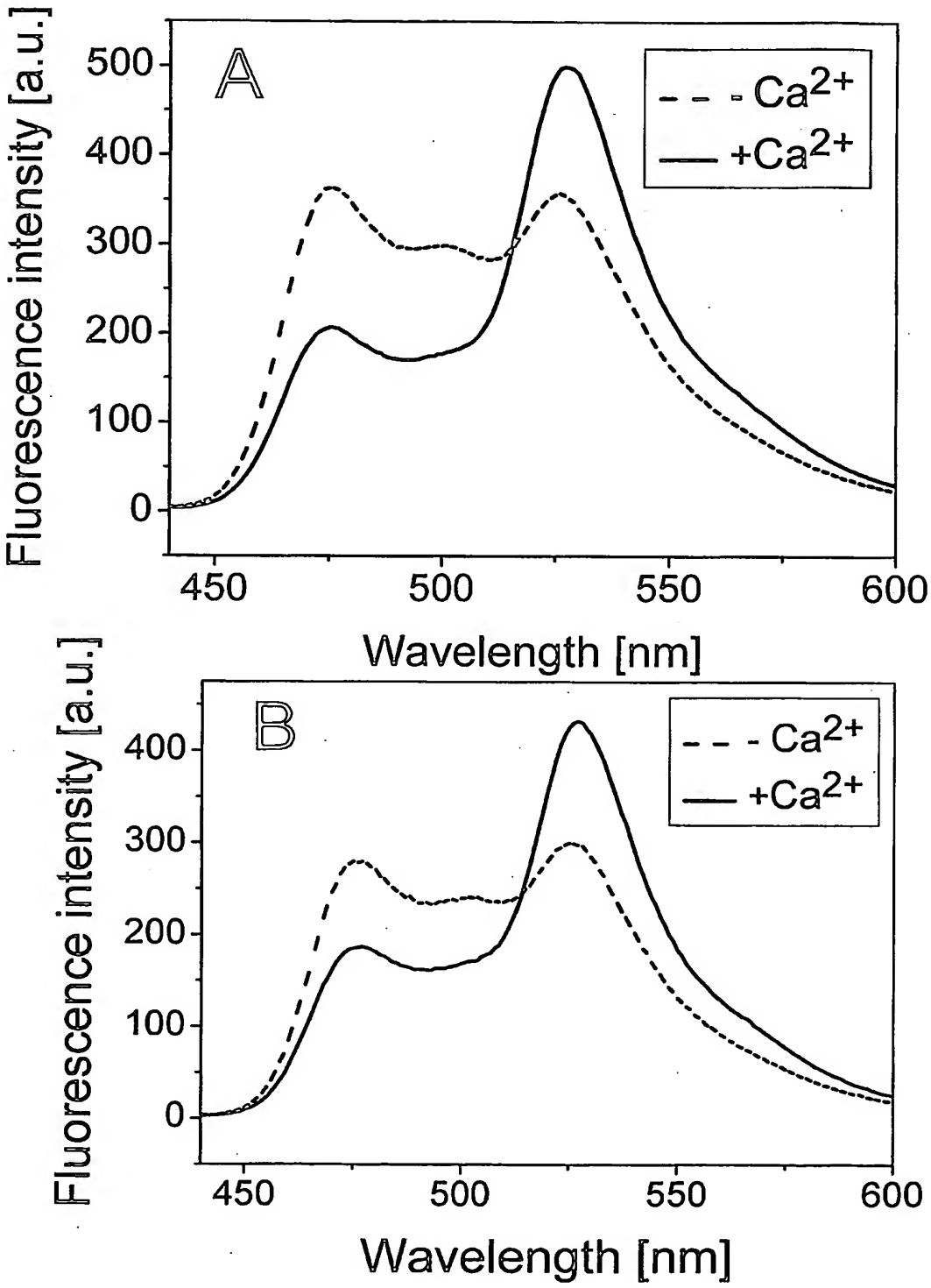

A
B
Fluorescence intensity [a.u.]
Wavelength [nm]
- - - Ca2+
—— +Ca2+
0
100
200
300
400
500
450
500
550
600

Fig. 4

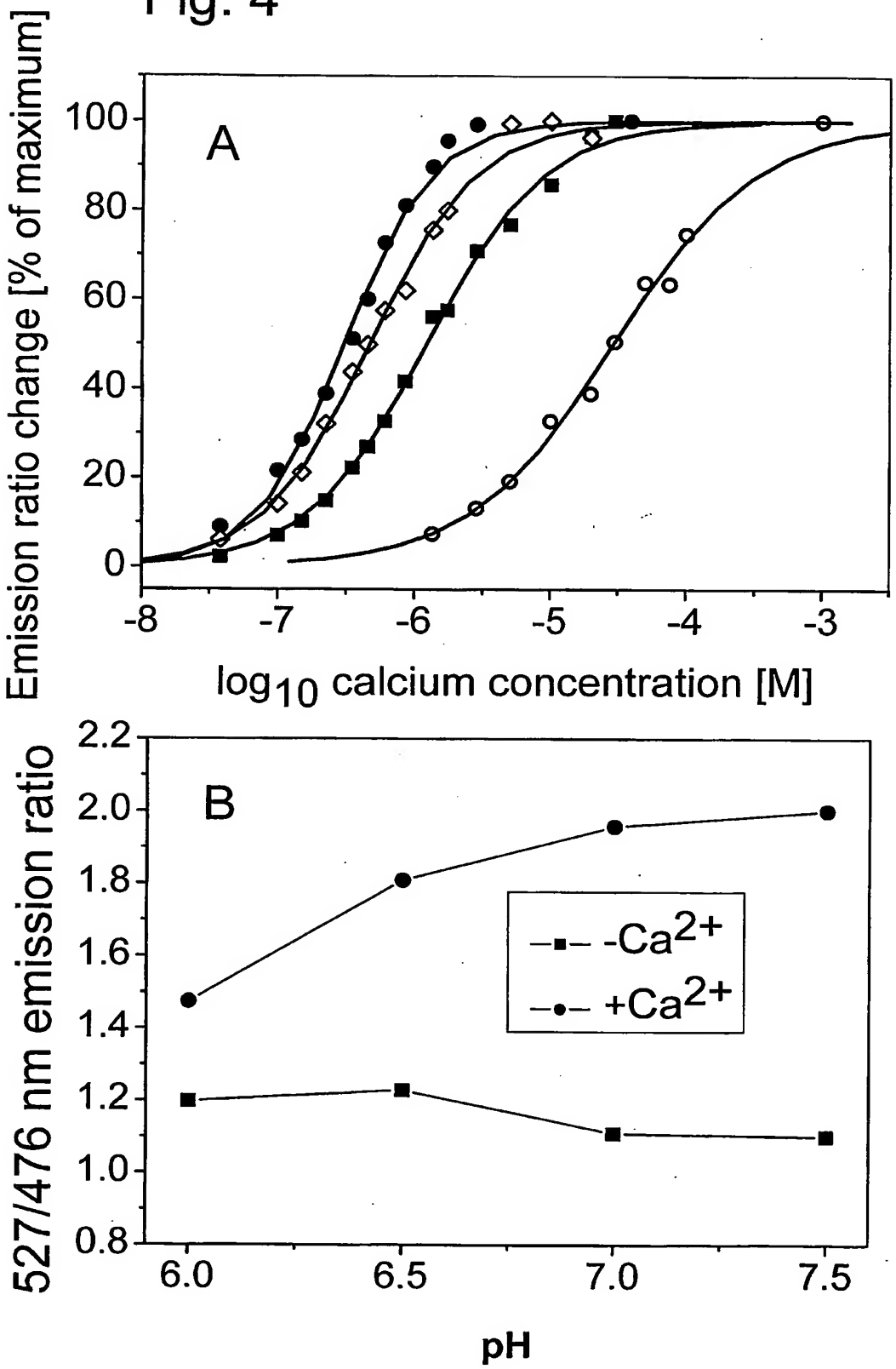

A
Emission ratio change [% of maximum]
100
80
60
40
20
0
-8
-7
-6
-5
-4
-3
log10 calcium concentration [M]
B
527/476 nm emission ratio
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
6.0
6.5
7.0
7.5
pH
-Ca2+
+Ca2+

Fig. 5

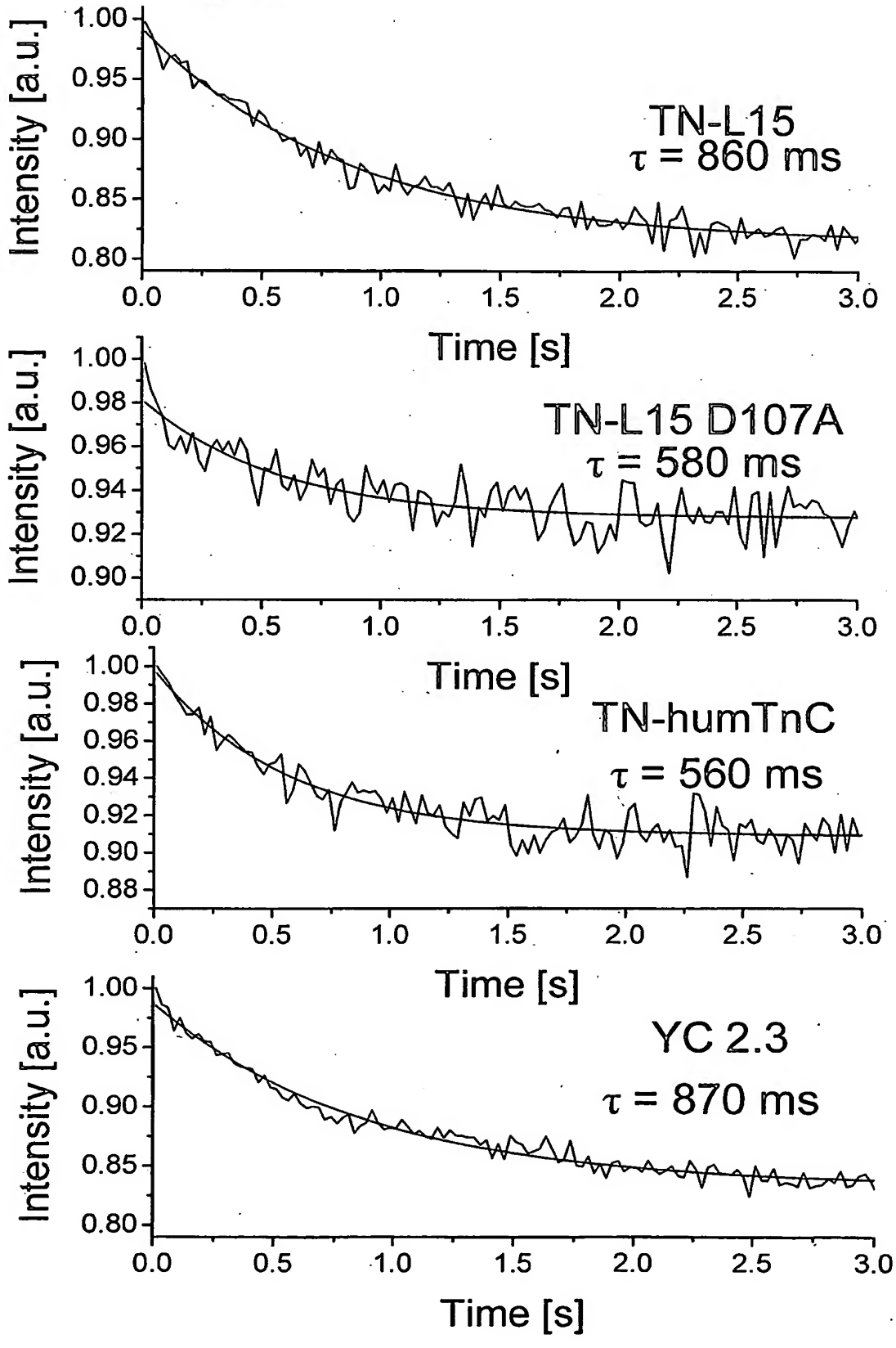

Intensity [a.u.]
1.00
0.95
0.90
0.85
0.80
TN-L15
τ = 860 ms
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Time [s]
Intensity [a.u.]
1.00
0.98
0.96
0.94
0.92
0.90
TN-L15 D107A
τ = 580 ms
Time [s]
Intensity [a.u.]
1.00
0.98
0.96
0.94
0.92
0.90
0.88
TN-humTnC
τ = 560 ms
Time [s]
Intensity [a.u.]
1.00
0.95
0.90
0.85
0.80
YC 2.3
τ = 870 ms
Time [s]

Fig. 6

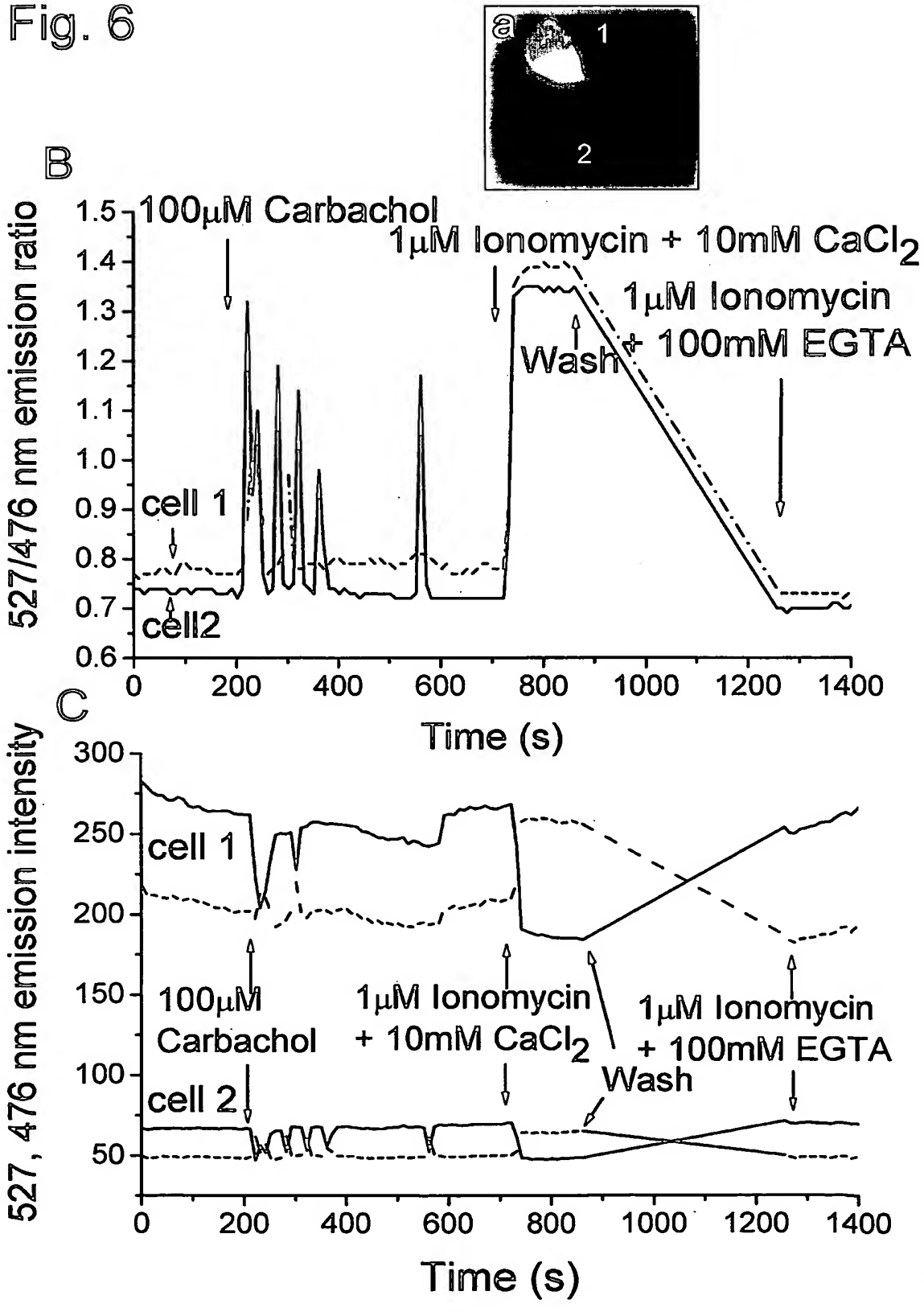

a
1
2
B
100µM Carbachol
1µM Ionomycin + 10mM CaCl2
1µM Ionomycin
+ 100mM EGTA
Wash
cell 1
cell2
527/476 nm emission ratio
Time (s)
C
cell 1
cell 2
100µM
Carbachol
1µM Ionomycin
+ 10mM CaCl2
Wash
1µM Ionomycin
+ 100mM EGTA
527, 476 nm emission intensity
Time (s)

Fig. 7

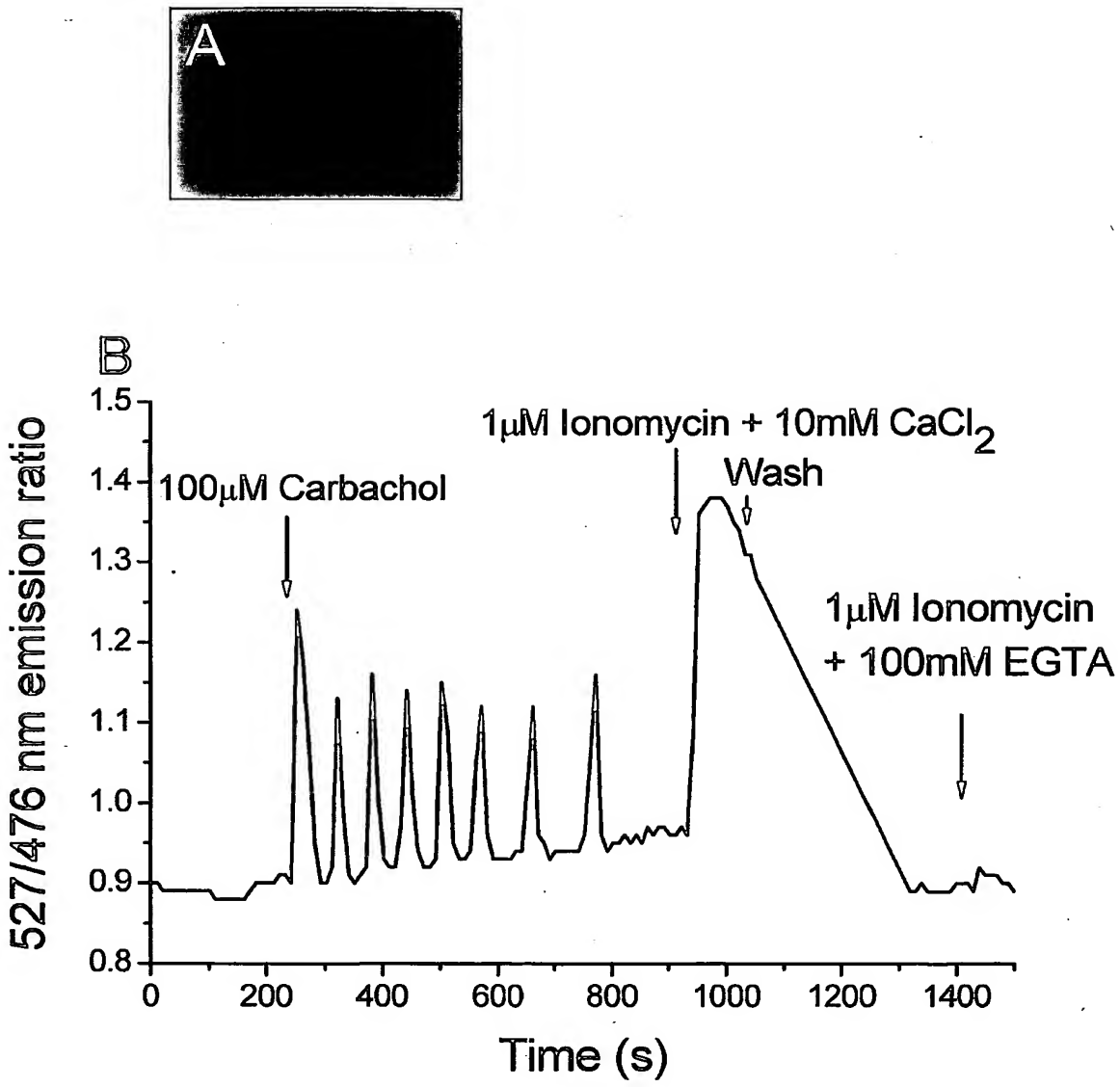

A
B
527/476 nm emission ratio
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
100µM Carbachol
1µM Ionomycin + 10mM CaCl2
Wash
1µM Ionomycin
+ 100mM EGTA
0
200
400
600
800
1000
1200
1400
Time (s)

Fig. 8

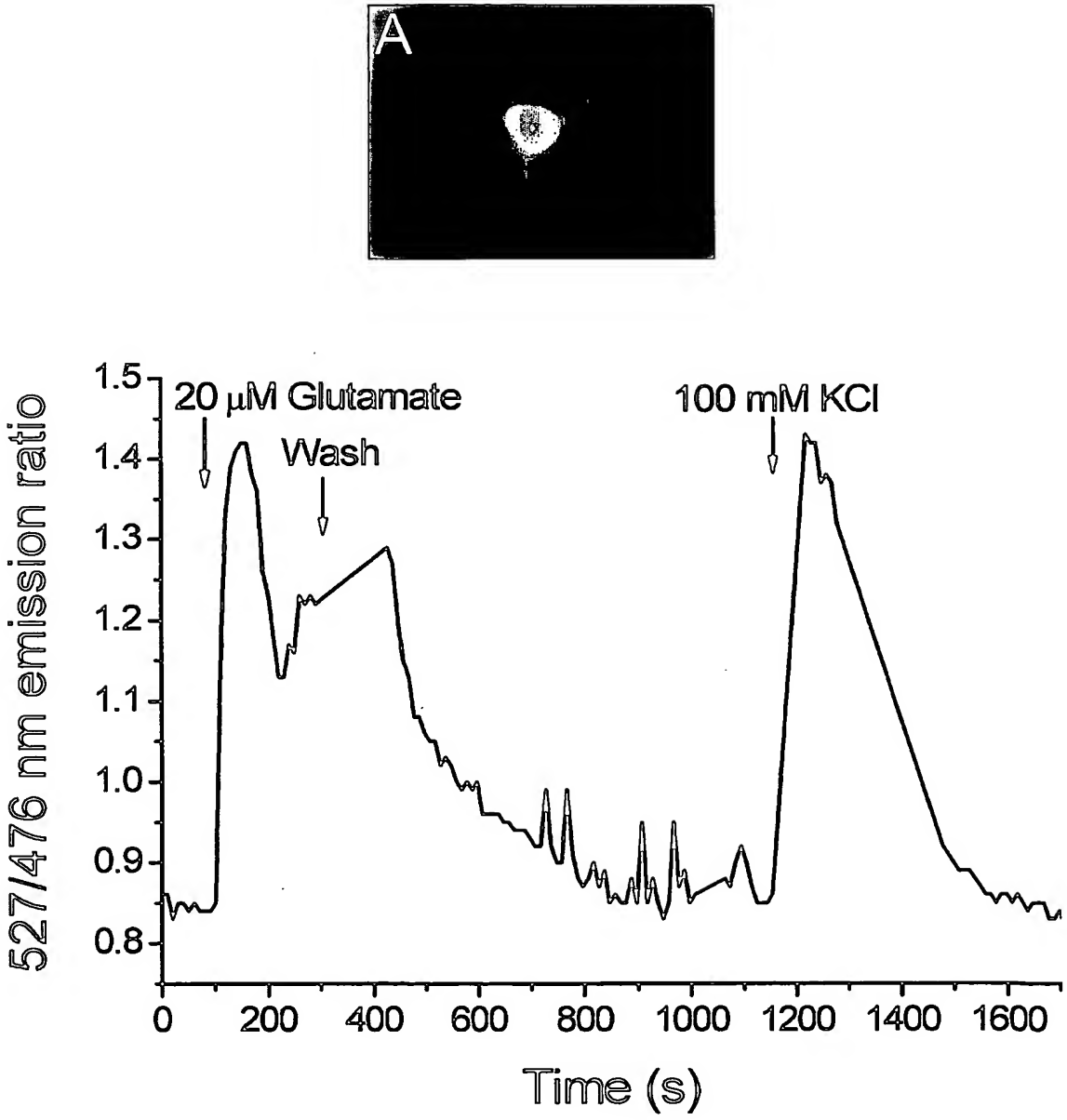
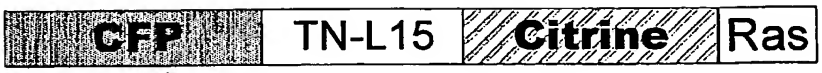
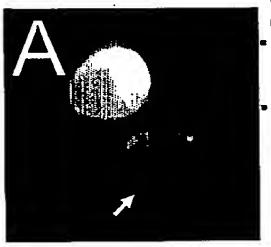
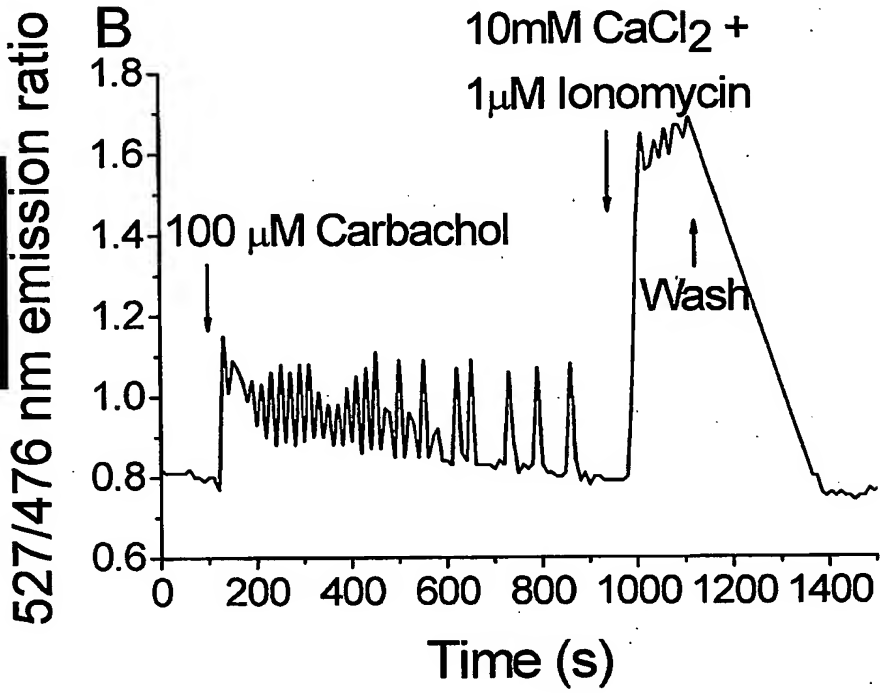

A
20 µM Glutamate
Wash
100 mM KCl
527/476 nm emission ratio
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0
200
400
600
800
1000
1200
1400
1600
Time (s)

Fig. 9


CFP
TN-L15
Citrine
Ras


A


B
10mM CaCl2 +
1μM Ionomycin
100 μM Carbachol
Wash
527/476 nm emission ratio
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0
200
400
600
800
1000
1200
1400
Time (s)


C

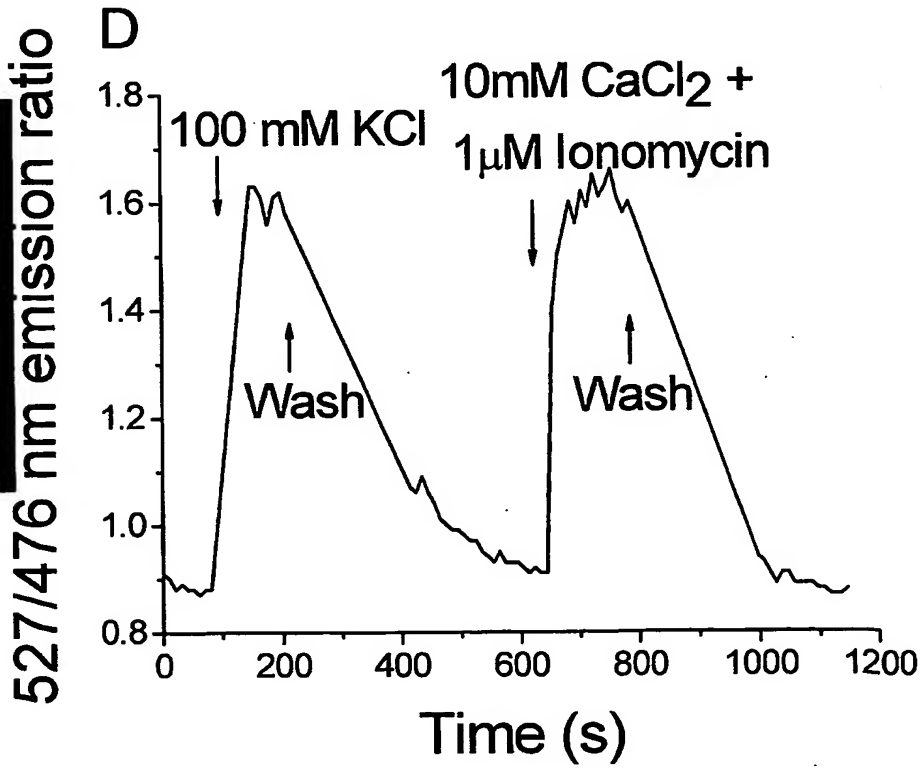

D
100 mM KCl
10mM CaCl2 +
1μM Ionomycin
Wash
Wash
527/476 nm emission ratio
1.8
1.6
1.4
1.2
1.0
0.8
0
200
400
600
800
1000
1200
Time (s)

Fig. 10

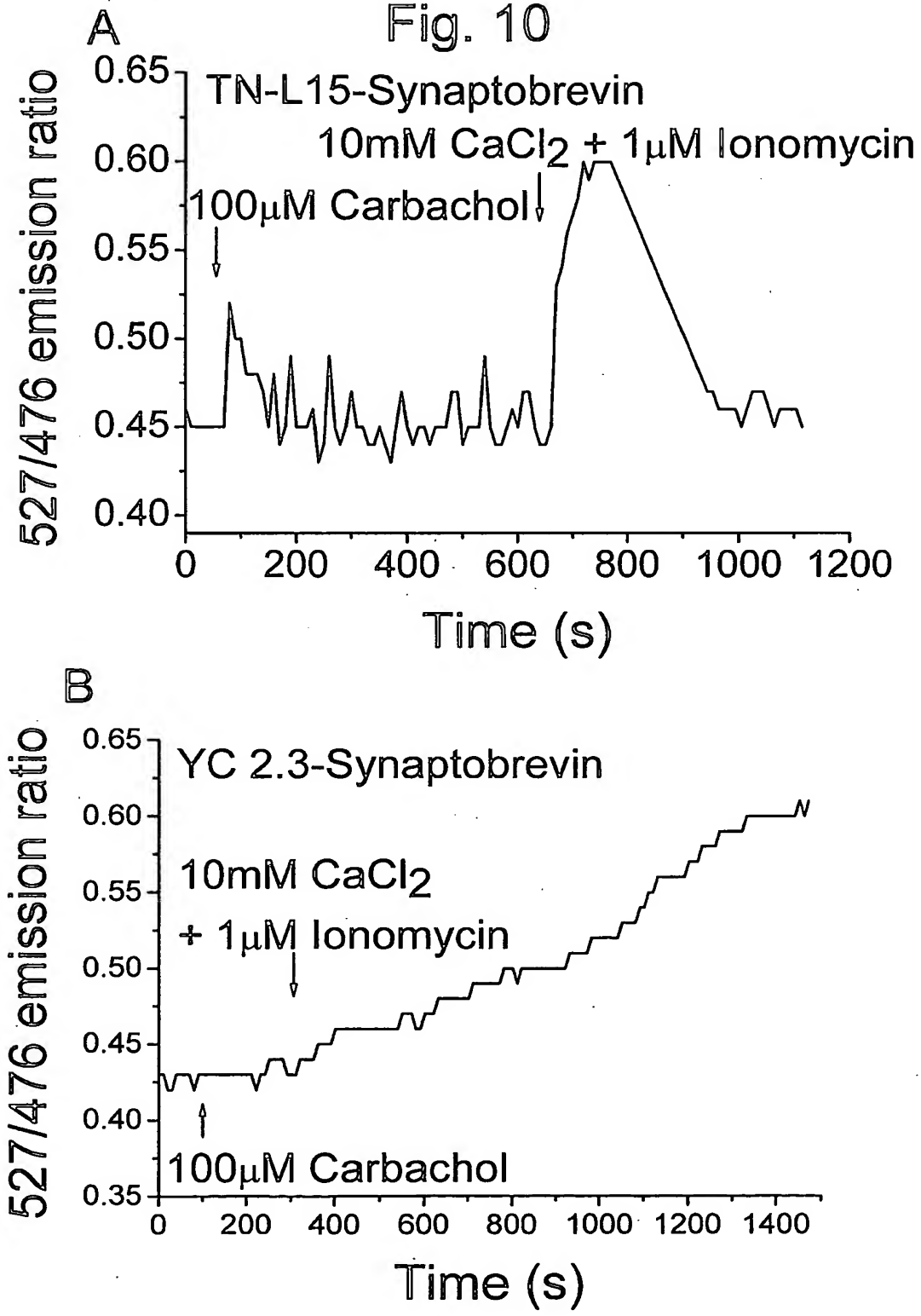

A
TN-L15-Synaptobrevin
10mM CaCl2 + 1µM Ionomycin
100µM Carbachol
527/476 emission ratio
0.65
0.60
0.55
0.50
0.45
0.40
0
200
400
600
800
1000
1200
Time (s)
B
YC 2.3-Synaptobrevin
10mM CaCl2
+ 1µM Ionomycin
100µM Carbachol
527/476 emission ratio
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0
200
400
600
800
1000
1200
1400
Time (s)

Fig. 11

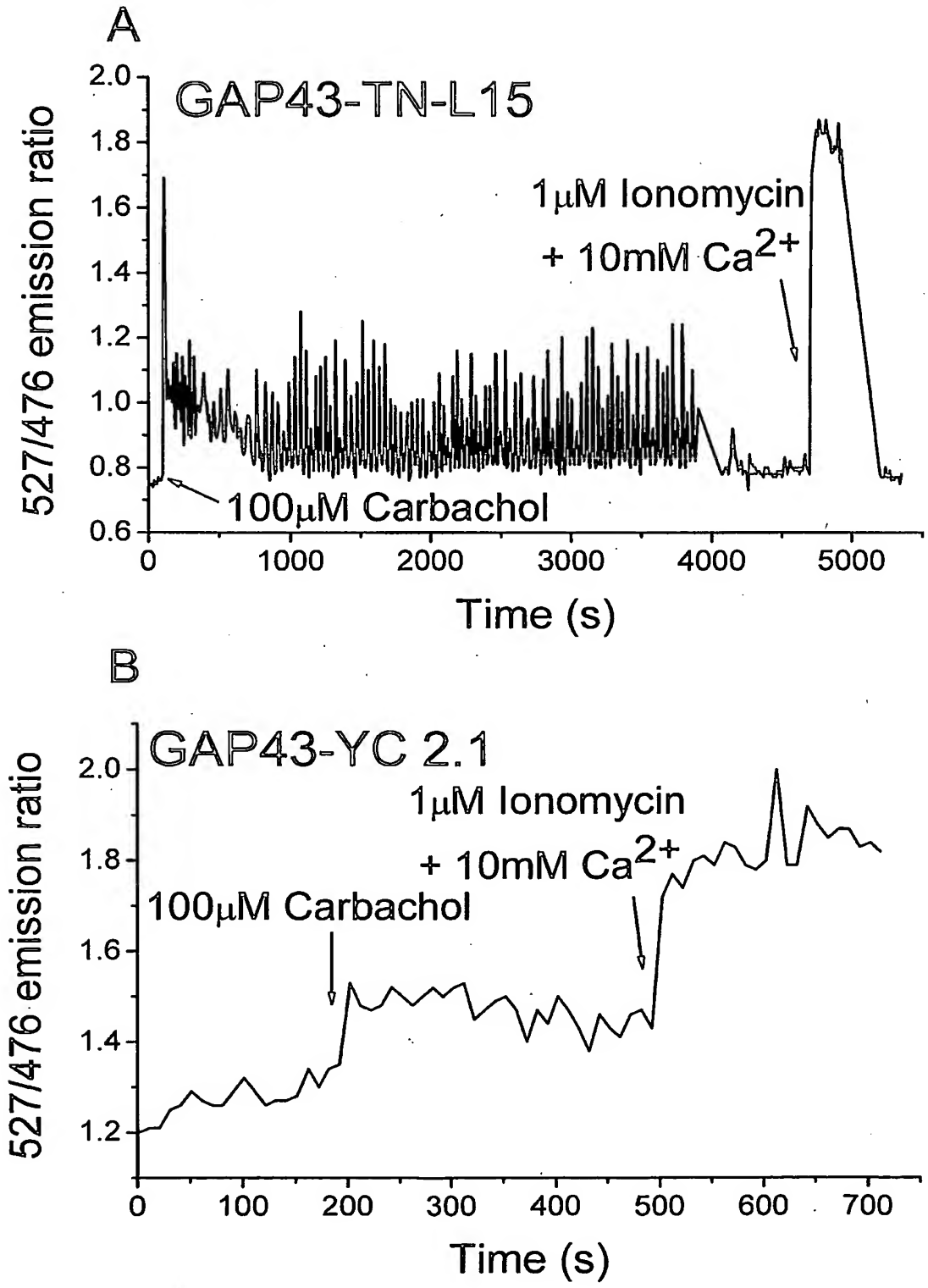

A
GAP43-TN-L15
527/476 emission ratio
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
1μM Ionomycin
+ 10mM Ca2+
100μM Carbachol
0
1000
2000
3000
4000
5000
Time (s)
B
GAP43-YC 2.1
527/476 emission ratio
2.0
1.8
1.6
1.4
1.2
1μM Ionomycin
+ 10mM Ca2+
100μM Carbachol
0
100
200
300
400
500
600
700
Time (s)

Fig. 12

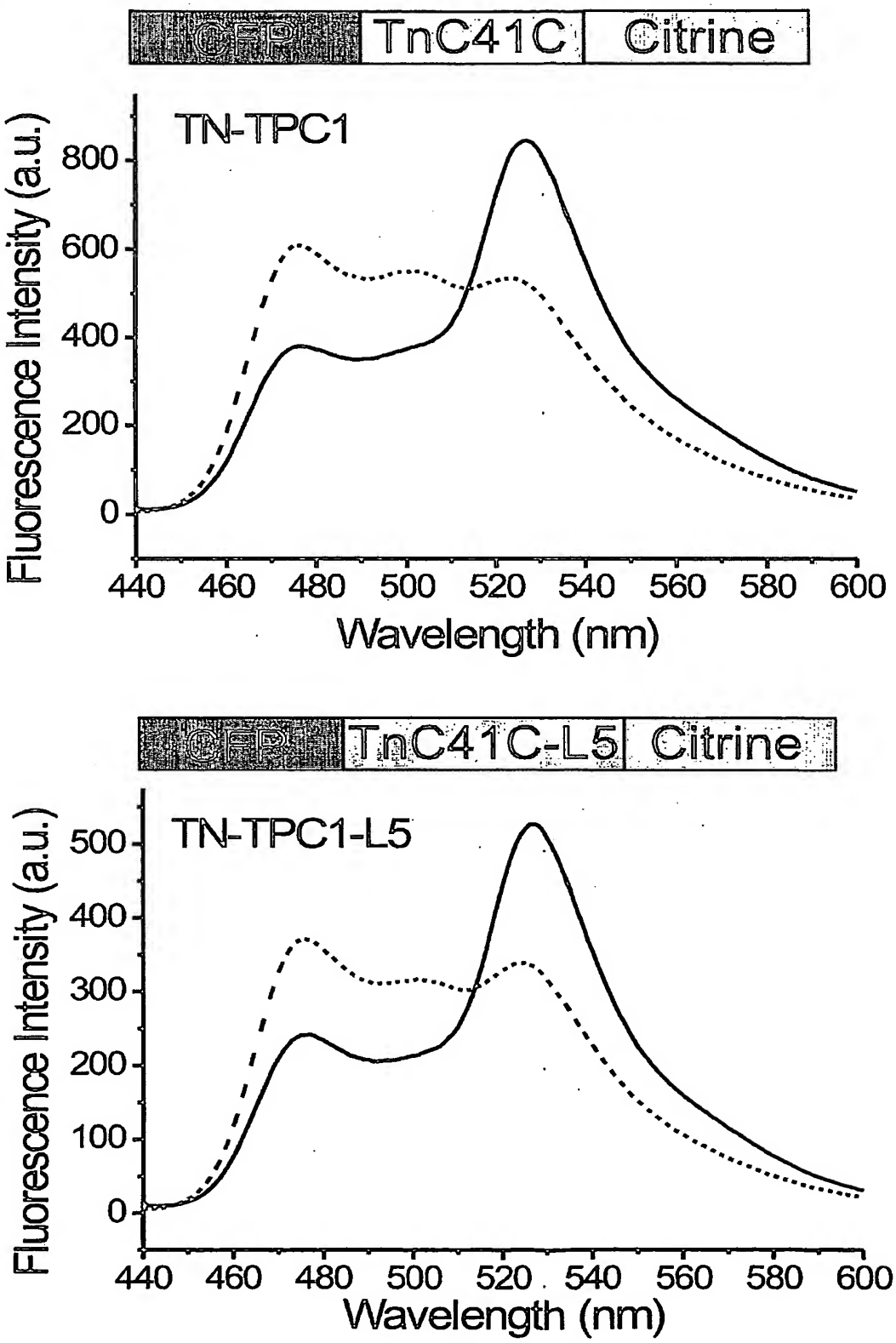

CFP
TnC41C
Citrine
TN-TPC1
Fluorescence Intensity (a.u.)
0
200
400
600
800
440
460
480
500
520
540
560
580
600
Wavelength (nm)
CFP
TnC41C-L5
Citrine
TN-TPC1-L5
Fluorescence Intensity (a.u.)
0
100
200
300
400
500
440
460
480
500
520
540
560
580
600
Wavelength (nm)

Fig. 13


Cop-Green
csTnC-L15
Phi-Yellow

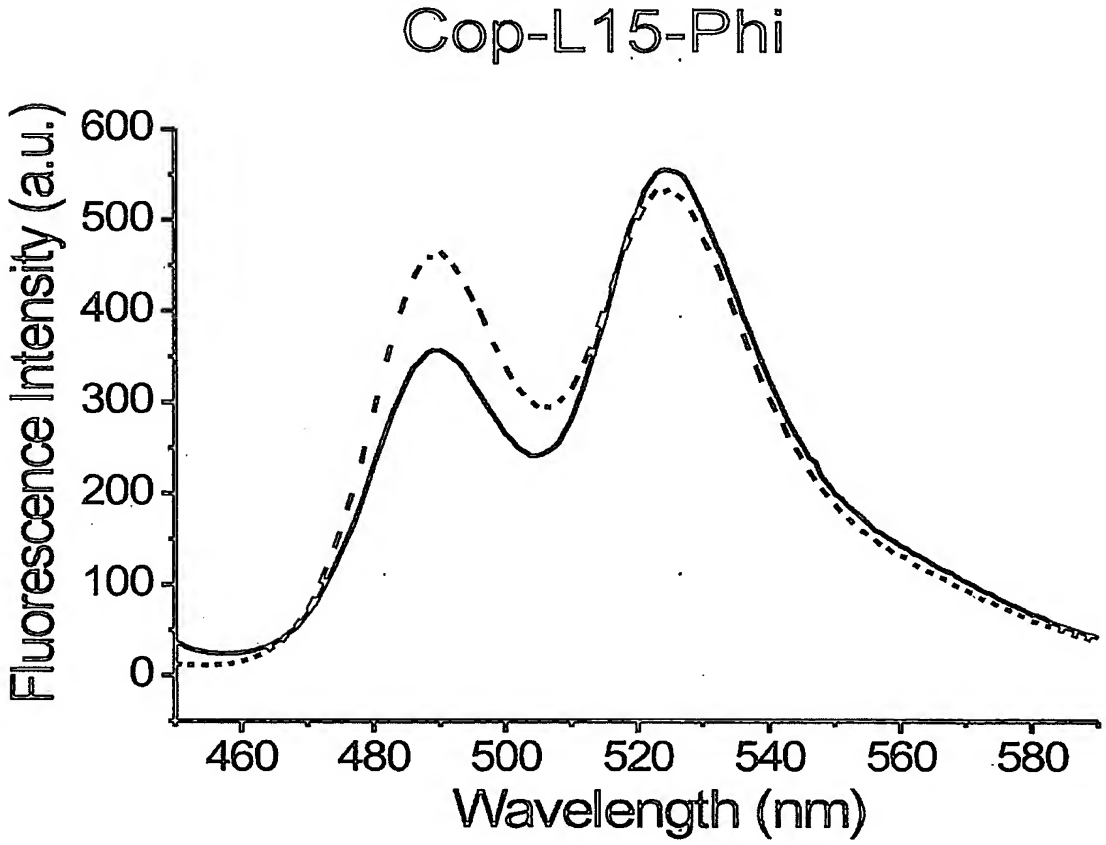
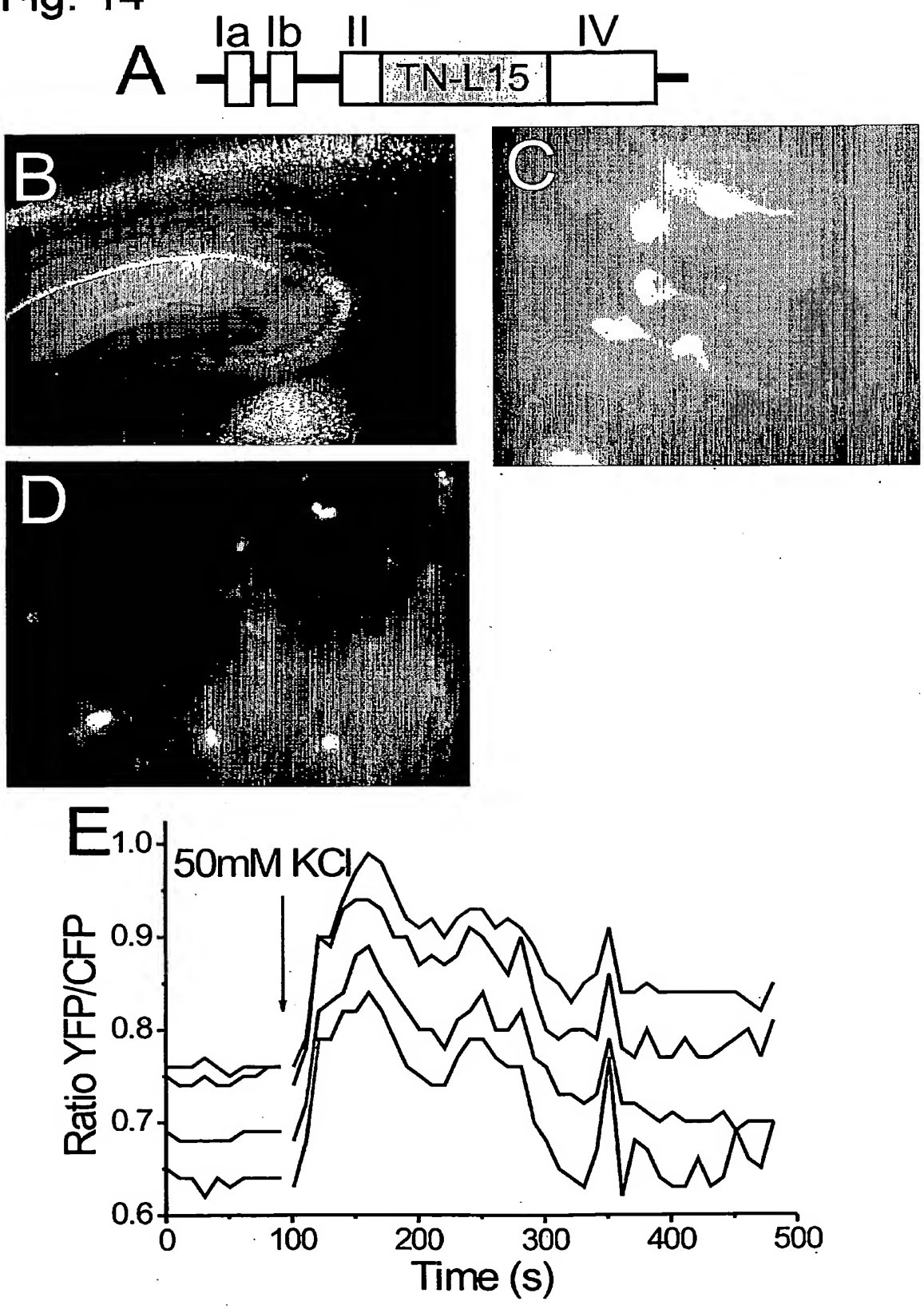

Cop-L15-Phi
Fluorescence Intensity (a.u.)
600
500
400
300
200
100
0
460
480
500
520
540
560
580
Wavelength (nm)

Fig. 14


A
Ia Ib II IV
TN-L15
B
C
D
E
50mM KCl
Ratio YFP/CFP
1.0
0.9
0.8
0.7
0.6
0
100
200
300
400
500
Time (s)